Development of a PBPK Model for Triadimefon and Triadimenol in Rats and Humans

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Abstract

A physiologically based pharmacokinetic (PBPK) model was developed for the conazole fungicide triadimefon (TFN) and its primary metabolite, triadimenol (TNL). Rat tissue:blood partition coefficients and metabolic constants were measured *in vitro* for both compounds. Kinetic time course data for parent and metabolite were collected from several tissues after intravenous administration of TFN to male Sprague Dawley rats. The model adequately simulated peak blood and tissue concentrations but failed to predict the observed slow terminal clearance of both TFN and TNL from blood and tissues. Two hypotheses were explored as possible explanations of this slow clearance: low capacity, high affinity protein binding of parent and metabolite in blood and tissues, and reverse metabolism of TNL to TFN in the liver. Model predictions were significantly improved in both hypothetical scenarios. The original model as well as both alternate models were extrapolated to humans using *in vitro* metabolic constants measured in human hepatic microsomes. Human equivalent doses (HEDs) were calculated for all three models for a rat NOAEL dose of 11.57 µmol/kg/day using area under the concentration curve (AUC) in brain and blood for TFN and TNL as dosimetrics. All dosimetric-based HEDs were above the oral reference dose of 0.11 µmol TFN/kg/day.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

Results

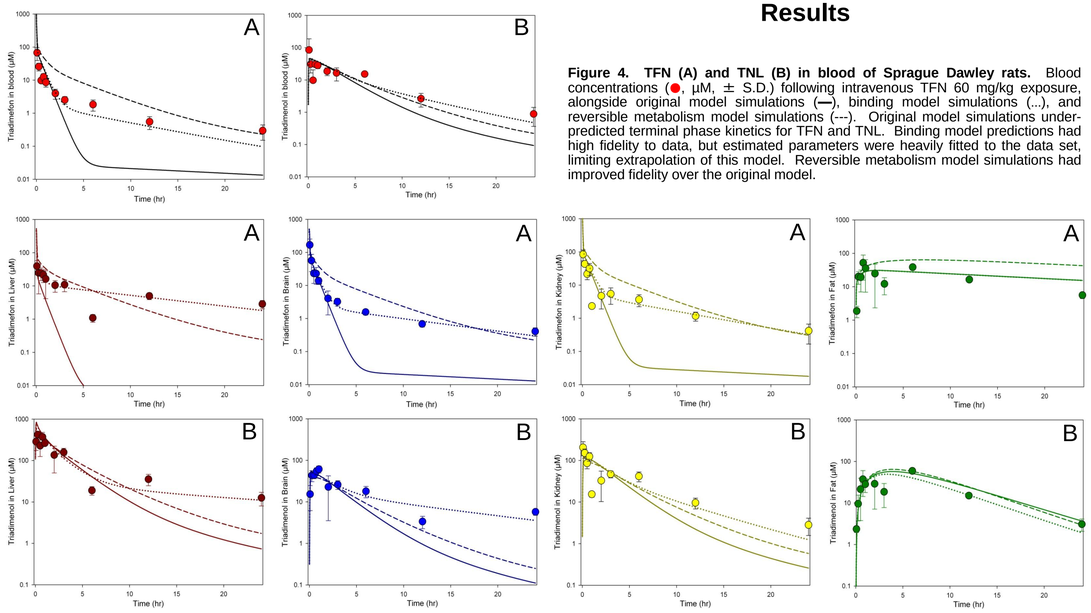

Figure 4. TFN (A) and TNL (B) in blood of Sprague Dawley rats. Blood concentrations (●, µM, ± S.D.) following intravenous TFN 60 mg/kg exposure, alongside original model simulations (—), binding model simulations (...), and reversible metabolism model simulations (---). Original model simulations under-predicted terminal phase kinetics for TFN and TNL. Binding model predictions had high fidelity to data, but estimated parameters were heavily fitted to the data set, limiting extrapolation of this model. Reversible metabolism model simulations had improved fidelity over the original model.

Figure 5. TFN (A) and TNL (B) in liver, brain, kidney, and fat of Sprague Dawley rats. Tissue concentrations (●, µM, ± S.D.) following intravenous TFN 60 mg/kg exposure, alongside original model simulations (—), binding model simulations (...), and reversible metabolism model simulations (---). For liver, brain, and kidney, original model simulations under-predicted terminal phase kinetics for TFN and TNL. Binding model predictions had high fidelity to data, but estimated parameters were heavily fitted to the data set, limiting extrapolation of this model. Reversible metabolism model simulations had improved fidelity over the original model. In fat, initial model predictions had high fidelity to data for both TFN and TNL. For TFN, the binding model made no appreciable difference to model simulations, while the reversible metabolism model over-predicted data. For TNL, all three models adequately predicted data with minor differences.

	Original		Binding		Reverse Metabolism	
Dose Metric	AUC (µmol/L·hr)	HED (µmol/kg/day)	AUC (µmol/L·hr)	HED (µmol/kg/day)	AUC (µmol/L·hr)	HED (µmol/kg/day)
TFN Blood	0.73	0.45	0.73	1.1	10.8	2.8
TFN Brain	0.70	0.45	0.99	0.64	10.4	2.8
TNL Blood	9.7	1.4	7.2	7.9	10.3	3.0
TNL Brain	11.4	0.32	25.7	0.78	12.2	3.0

Table 1. HEDs for NOAEL exposure in SD rat. Required HEDs were all above the human oral reference dose of 0.1157 µmol/kg/day.

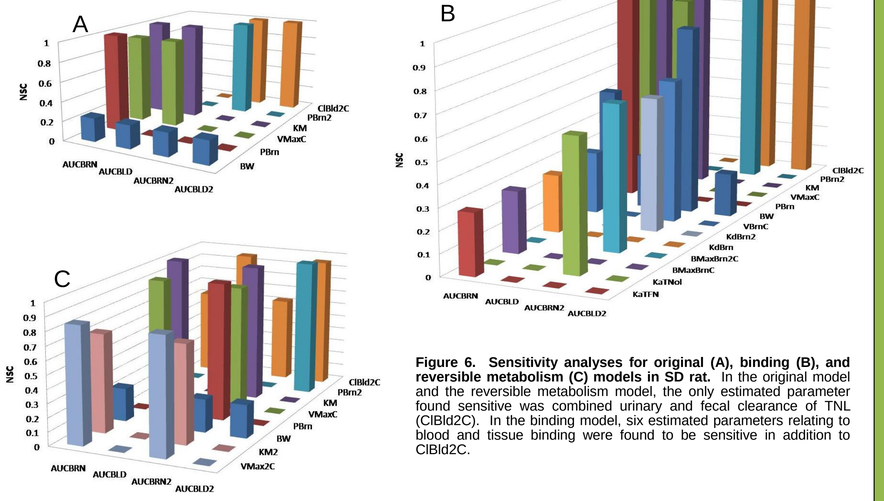

Figure 6. Sensitivity analyses for original (A), binding (B), and reversible metabolism (C) models in SD rat. In the original model and the reversible metabolism model, the only estimated parameter found sensitive was combined urinary and fecal clearance of TNL (ClBld2C). In the binding model, six estimated parameters relating to blood and tissue binding were found to be sensitive in addition to ClBld2C.

Methods

- **EXPERIMENTAL**
  - Microsomal **metabolism** studies
    - Michaelis Menten regression of concentration vs. time data on TFN depletion and TNL formation
      - Male SD rat (Crowell et al., 2010)
      - Male Human
  - *In vitro* **partition coefficient** measurement
    - Method adapted from Jepson et al., 1994
    - Male SD rat tissues - blood, liver, brain, kidney, fat
  - *In vivo* **pharmacokinetic** study in male SD rats
    - Intravenous administration of 60 mg/kg TFN
    - Terminal collection - blood, liver, brain, kidney, fat
- **COMPUTATIONAL**
  - Initial model development (**Figure 1)**
  - Model refinement
    - Blood and tissue binding (**Figure 2)**
    - Reversible metabolism (**Figure 3**)
- **APPLICATION**
  - Dose metric calculation from critical study NOAEL
    - Oral exposure to 11.57 µmol/kg/day in SD rats
    - 12 hr constant intake per day, to steady state
    - AUC_{BLOOD} and AUC_{BRAIN} for TFN and TNL
  - Extrapolation to humans
    - 3 x 30 minute meals per day, to steady state
    - Human equivalent doses (HEDs) for each metric
    - Compared to oral RfD of 0.1157 µmol/kg/day
- **SENSITIVITY ANALYSES** (**Figure 7)**
  - Normalized sensitivity coefficients (NSCs) calculated:

$$NSC = \frac{(Output_{1.1} - Output)/Output}{(Parameter_{1.1} - Parameter)/Parameter}$$

  - NSCs >0.15 relevant, >1.0 capable of amplifying error

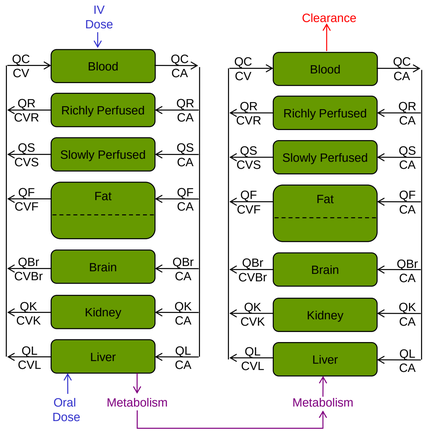

Figure 1. PBPK model structure for TFN and its metabolite TNL. Compartments represent physiological tissues and organs. Model code consists of systems of differential equations describing the movement of chemical into and out of each compartment (arrows). Routes of administration are shown in **blue**, metabolism is shown in **purple**, and routes of excretion are shown in **red**.

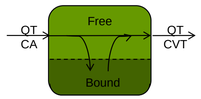

Figure 2. Detailed schematic of macromolecular binding. Binding of parent and metabolite in blood and tissues was explored as a possible model refinement. As chemical moves through a compartment according to perfusion rates, some portion becomes bound in a sub-compartment. Chemical moves into and out of this sub-compartment according to estimated association (K_A) and dissociation K_D) constants; the maximum amount bound was dictated by an estimated binding capacity (B_{MAX}, µmol)

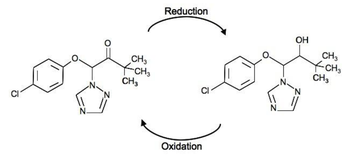

Figure 3. Reversible metabolism of TFN and TNL. Bidirectional metabolism was explored as a possible model refinement. 11β-HSD1, responsible for TFN reduction, is reversible and catalyzes interconversion of native substrates cortisone and cortisol. Kinetic parameters for the oxidation of TNL to TFN were derived from values for cortisol oxidation to cortisone (Diederich et al. 2000). All metabolism was assumed to take place in the liver compartments.

Conclusions

- Simulations by the original model insufficiently predicted terminal phase kinetics of TFN and TNL disposition.
  - For TFN, data indicated lingering concentrations near 1 µM for most tissues, while model predictions were markedly lower.
  - For TNL, model predictions were marginally better, but still under-predicted observed data.
- The binding model provided marked improvements to simulations, but required many estimated parameters.
  - Heavily reliant on single pharmacokinetic data set.
  - Unique binding parameters for each compartment constitutes a biologically unlikely explanation.
  - Sensitivity analyses found parameters relating to binding to be sensitive in addition to initial model sensitive parameters.
- The reversible metabolism model had improved fidelity to the observed data.
  - Required no additional estimated parameters.
  - Increased fidelity is anticipated upon experimental measurement of TNL oxidation .
  - Sensitivity analyses found the oxidation kinetic parameters to be sensitive in addition to those parameters sensitive in the original model.
- Upon extrapolation of all three models to humans, predicted HEDs to the critical rat NOAEL were all above the oral RfD for TFN.
  - Models not validated in humans due to lack of sufficient data.
  - Many HEDs for original and binding models were within an order of magnitude of the RfD, indicating a need for further investigation.

Future Directions

- Test model generated hypotheses experimentally
  - Measure oxidation rates for TNL in liver and kidney microsomes
  - Investigate the capacity for macromolecular binding in rat blood and tissues
- As data becomes available, validate the model for use in humans
  - NHANES
  - Occupational data

References

Crowell, S.R. et al. (2010). *Toxicology Letters* 193, 101 – 107.

Diederich, S. et al. (2000). *European Journal of Endocrinology* 142, 200 – 207.

Jepson, G.W. et al. (1994). *Fundamental and Applied Toxicology* 22, 519 – 524.